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TITLE: Regulation of Drug Sensitivity by Functional Status of p53 in Human Prostate Cancer

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13. ABSTRACT (Maximum 200 Words) Effective treatment of prostate cancer requires a better understanding of drug resistance mechanism and better therapeutic strategy. We previously found that flutamide, and anti-androgen commonly used in the treatment of prostate cancer, was a substrate of MRP1, a transmembrane phosphoprotein. During this grant period we further characterized the properties of flutamide transport by MRP1. We demonstrated that transport of flutamide and hydroxyflutamide by MRP1 was ATP-dependent, further strengthening the conclusion that flutamide and its active metabolite hydroxyflutamide were transported by MRP1. In addition, our preliminary data showed that nilutamide, a flutamide derivative that is currently used as second line hormone therapy for prostate cancer, was not a substrate of MRP1, suggesting that nilutamide may still be used in the treatment of prostate cancer with the overexpression of MRP1. Using the ELISA assay established in our laboratory, we screened a set of structurally related compounds for their activity of stabilizing p53. Among the compounds tested, we found that promazine, chlorpromazine and trans-flupenthixol were able to stabilize p53. In our LVCaP cell model that harbors a temperature-sensitive p53 mutant, the compounds that possess p53-stabilizing effect appear to be effective in sensitizing drug resistant cells to chemotherapeutic drugs.				
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INTRODUCTION

The treatment of recurrent prostate cancer is unsatisfactory. Following androgen-ablation therapies even the most active chemotherapeutic drugs produce meaningful responses in less than 50% of patients. Flutamide is a non-steroidal anti-androgen that acts as a competitive inhibitor of dihydrotestosterone for the androgen receptor (1) and is used as a part of initial treatment. Flutamide and its active metabolite hydroxyflutamide block expression of genes whose promoters contain androgen response elements and also prevent androgen-dependent stabilization of the androgen receptor (2). Although >50% of patients initially respond to androgen deprivation most patients relapse within a median of 12 to 18 months at which time they are resistant to further hormonal therapy (3). Re-treatment with anti-androgen therapy or chemotherapy has not been shown to significantly improve the overall survival of 2-3 years (4). Several factors may contribute to resistance following relapse from androgen-deprivation therapy. These include: increased affinity of the receptor for testosterone due to mutation of the androgen receptor (5, 6), increased androgen-receptor mediated transcription through activation of PKA (7), and decreased apoptosis due to mutations in p53 (8). We found that progression from benign prostate epithelium to high-grade prostate cancer correlated with the expression of drug-resistance proteins (9). Our results indicated that overexpression of MRP1 correlated with expression of mutant p53 (9), and that the expression of MRP1 was negatively regulated by the wild-type protein (10). MRP1 is a 190,000 kDa membrane protein belonging to the ATP binding cassette family, and can actively transport negatively charged substrates out of the cells (11-13). Conjugation or co-transport of substrates with glutathione, glucuronides, or sulfates can enhance transport by MRP1 (14, 15). Substances that bind MRP1 in the presence of glutathione include leukotrienes, doxorubicin, and vincristine (15, 16). We previously reported that anti-androgen drug flutamide and its metabolite hydroxyflutamide are MRP1 substrates (17). During this grant period, we further studied the transport characteristics of flutamide in human cancer cells. We have also screened a set of phenothiazine or thioxanthene compounds for their activity of stabilizing or rescuing p53, and sensitizing drug resistant cancer cells to chemotherapeutic drugs.

BODY

Task 1 To determine the role of MRP1 and MAP-4 in resistance to hormonal and chemotherapy.

During the last grant period we further examined the properties of transport of flutamide and its metabolite, hydroxyflutamide, by MRP. We found that MRP1 transport of flutamide and hydroxyflutamide were ATP-dependent (Appendix, Figures 1 and 2). Figure 1 shows that treatment of drug resistant cancer cell lines (KB4D-10 and PC-3-ADR) overexpressing MRP1 with sodium azide and 2-deoxy-D-glucose inhibited the transport of flutamide (A) and hydroxyflutamide (B). In addition, at 4°C the transport of those drugs was also reduced (Figure 2). These results indicate that MRP1 transports flutamide and hydroxyflutamide in an ATP-dependent manner.

Nilutamide is a flutamide derivative that shows efficacy in some flutamide-refractory patients, and is currently used as second line hormone therapy for prostate cancer (18). To determine whether or not nilutamide is a substrate for MRP1, we tested the effect of nilutamide on the accumulation of hydroxyflutamide. Figure 3 shows that nilutamide did not alter the steady-state accumulation of hydroxyflutamide, suggesting that nilutamide is transported by MRP1. Therefore, nilutamide may still be used in the treatment of prostate cancers with overexpression of MRP1. Currently, radio-labeled nilutamide is not available and we are not able to measure the nilutamide transport directly.

Task 2 To determine whether pharmacological stabilization of wild-type p53 and rescue of mutant p53 can suppress the expression of MRP1 and MAP-4 and sensitize cells to chemotherapy.

We investigated the effect of CP-31398, a compound known to stabilize wild-type p53 and rescue mutant p53 (19), on the sensitivity to MRP1-transportable chemotherapeutic drugs. Figure 4 shows that pre-treatment of prostate cancer cell line harboring temperature-sensitive p53 mutant with CP-31398 could sensitize the cells to vincristine, a MRP1-transportable drug.

Using the ELISA assay developed by our laboratory and described in the last progress report, we have screened 6 compounds that share structure similarity to CP-31398 for their activity of stabilizing or rescuing p53. These compounds are: promazine, chlorpromazine, imipramine, *trans*-flupenthixol, fluphenazine, and trifluorperazine (chemical structures are shown in Figure 5). Among these 6 compounds, promazine, chlorpromazine and *trans*-flupenthixol were found to possess activity of stabilizing p53 (Figure 6). This effect was confirmed by measurement of p21 expression (Figure 7).

KEY RESEARCH ACCOMPLISHMENTS

- We demonstrated that transport of anti-androgen drug flutamide and its active metabolite, hydroxyflutamide, by MRP1 is ATP-dependent.
- Among the compounds we screened, we found that promazine, chlorpromazine and *trans*-flupenthixol are able to stabilize p53.
- Our studies indicate that p53-stabilizing or rescuing compounds can sensitize MRP1-overexpressing cancer cells to chemotherapeutic drugs.

REPORTABLE OUTCOMES

Manuscript

Grzywacz MJ, Yang JM, Hait WN: Effect of Overexpression of the Multidrug Resistance Protein (MRP1) on the Transport of the Anti-androgen Flutamide. *Cancer Res* 63(10): 2492-2498, 2003.

Abstracts

Grzywacz MJ, Yang JM, Hait WN: The Active Metabolite of Flutamide, 2-hydroxyflutamide, is a substrate for MRP1. *Proc Amer Assoc Cancer Res* 44: 3685, 2003.

Degree obtained that are supported by this award

This award is supporting the research activities of a third-year graduate student (Ph.D. candidate).

CONCLUSIONS

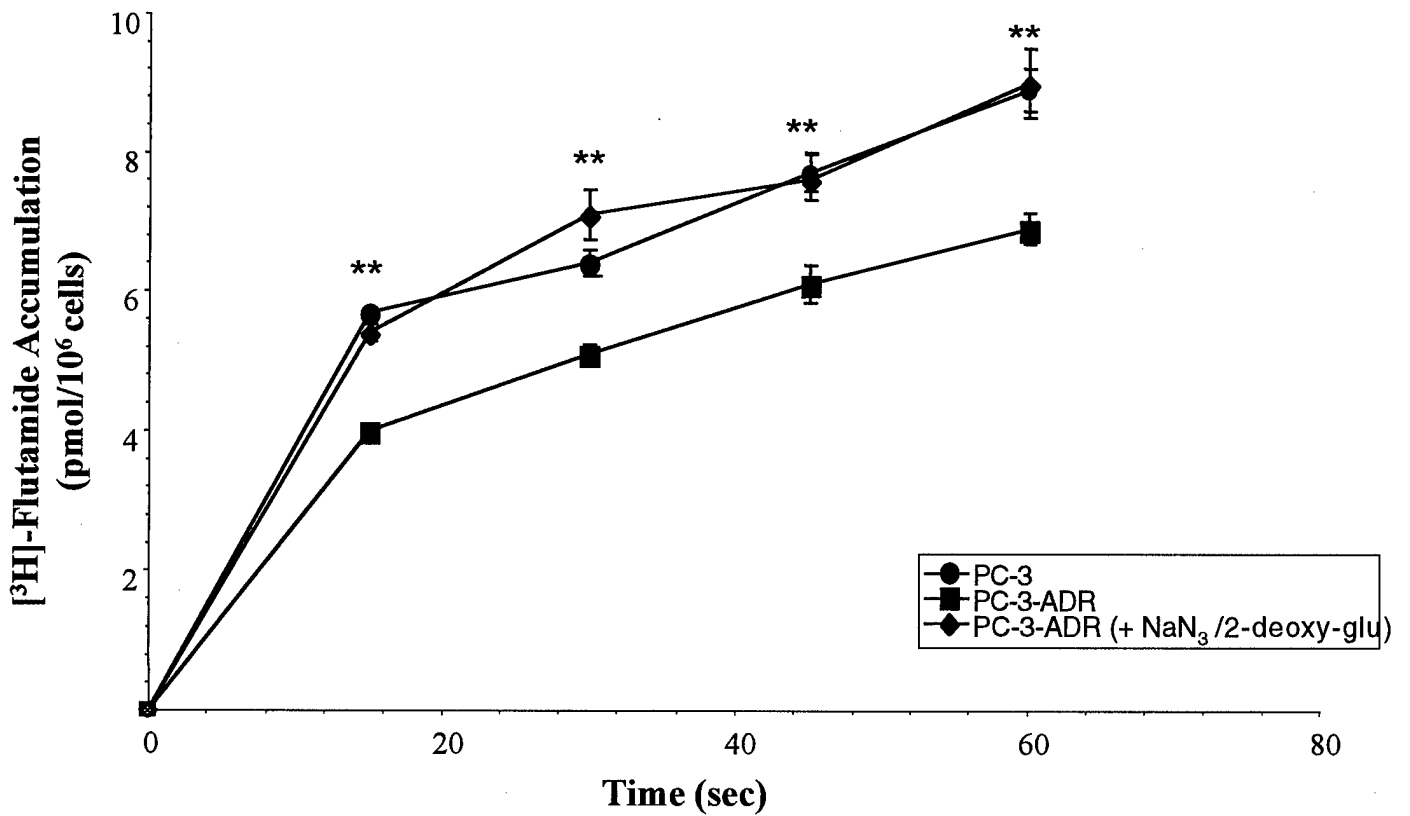
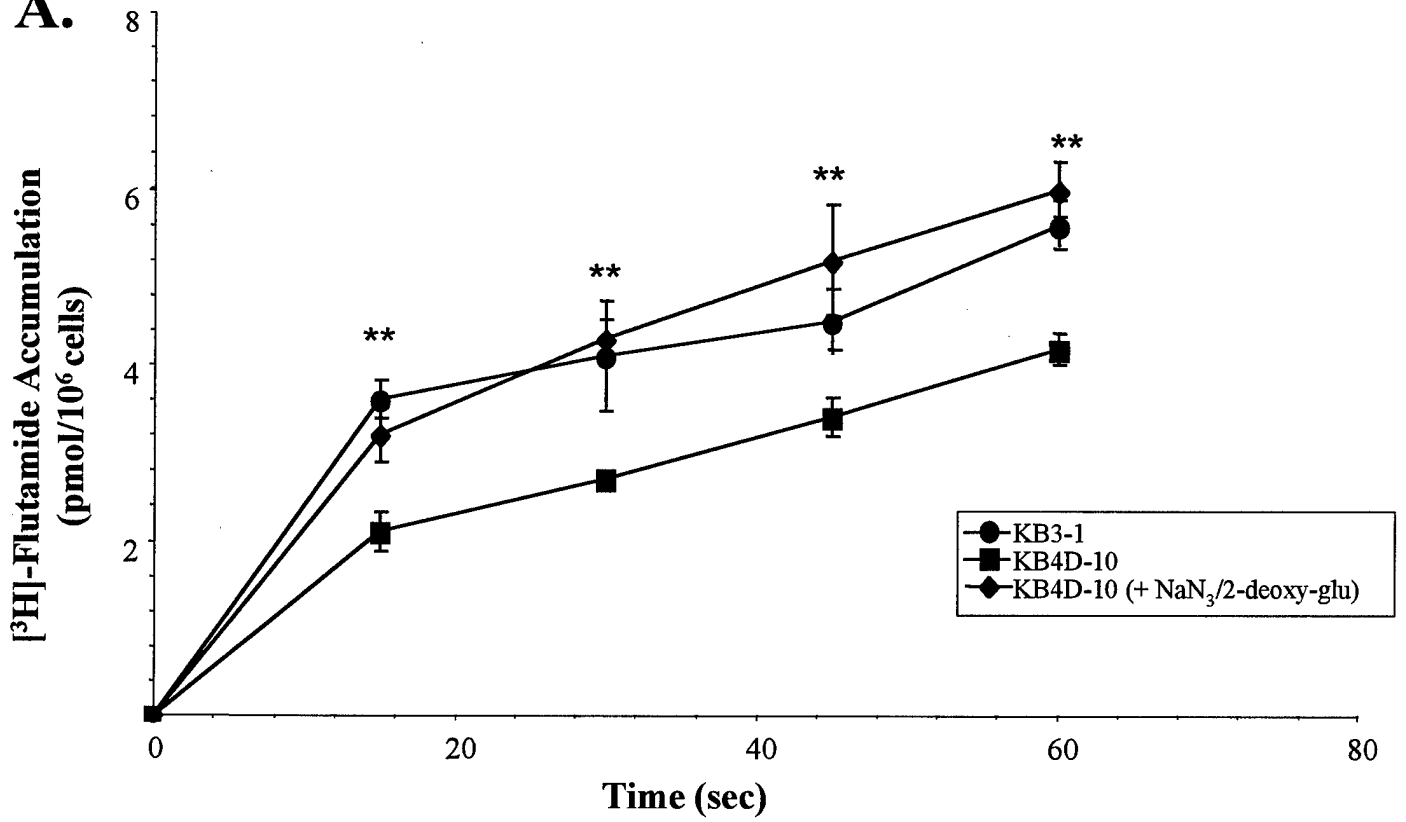
Our studies demonstrate that transport of flutamide and hydroxyflutamide by MRP1 is ATP-dependent. Also, we have identified three phenothiazine or thioxanthene compounds that possess p53-stabilizing activity. Our results indicate that compounds able to stabilize p53 can sensitize cancer cells to chemotherapeutic drugs that are transported by MRP1, and may have clinical implications for the treatment of drug resistant prostate cancers.

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APPENDICES

A.**Fig. 1A**

B.

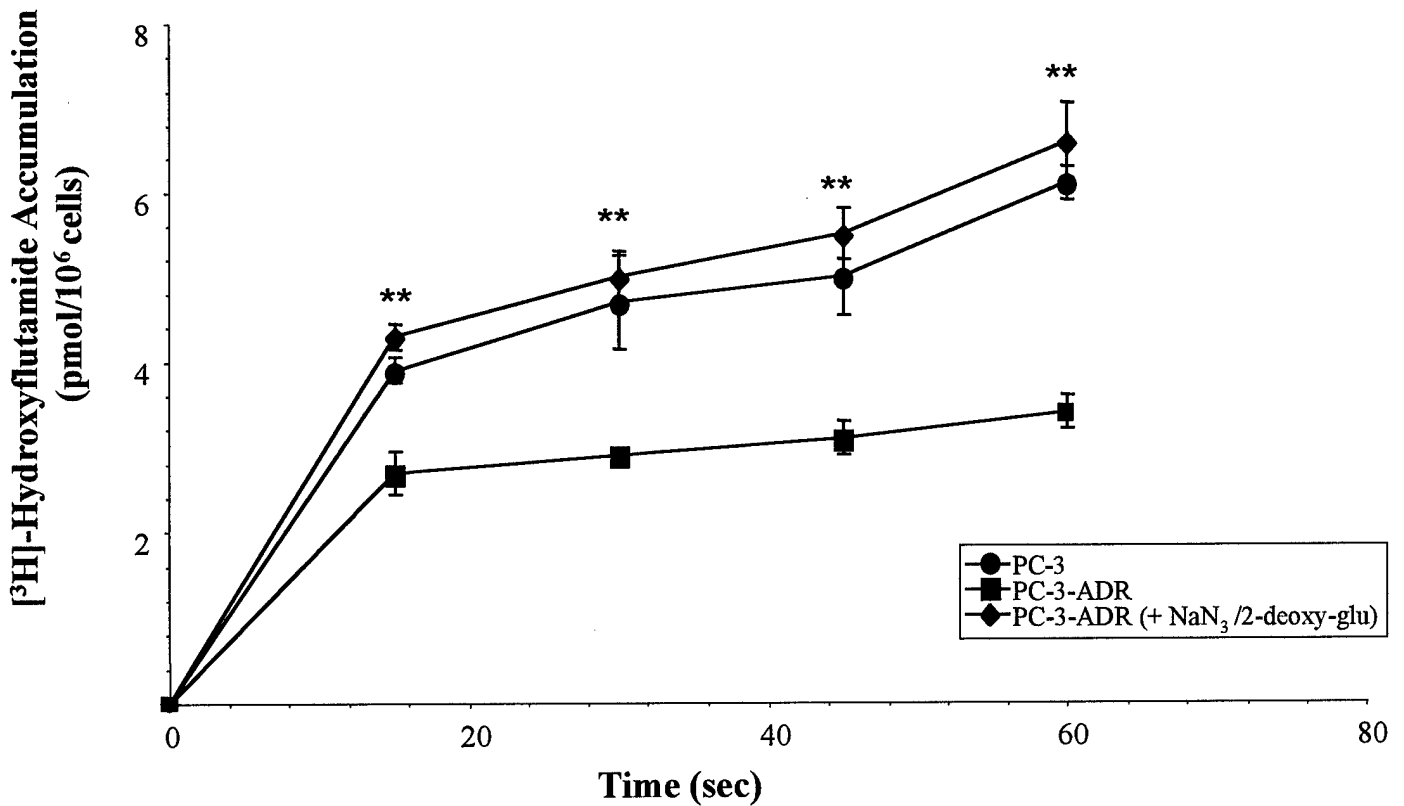
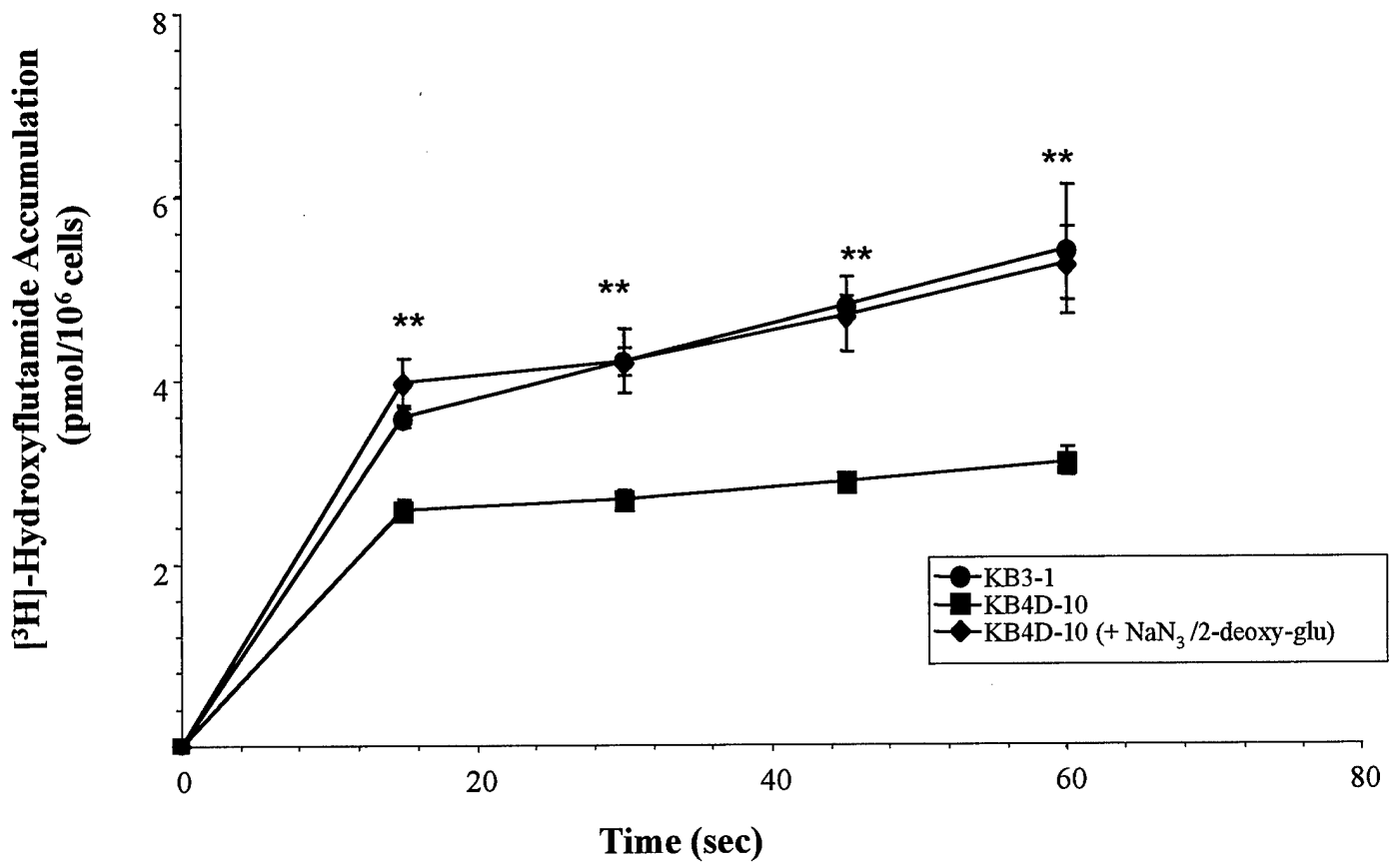


Fig. 1B

A.

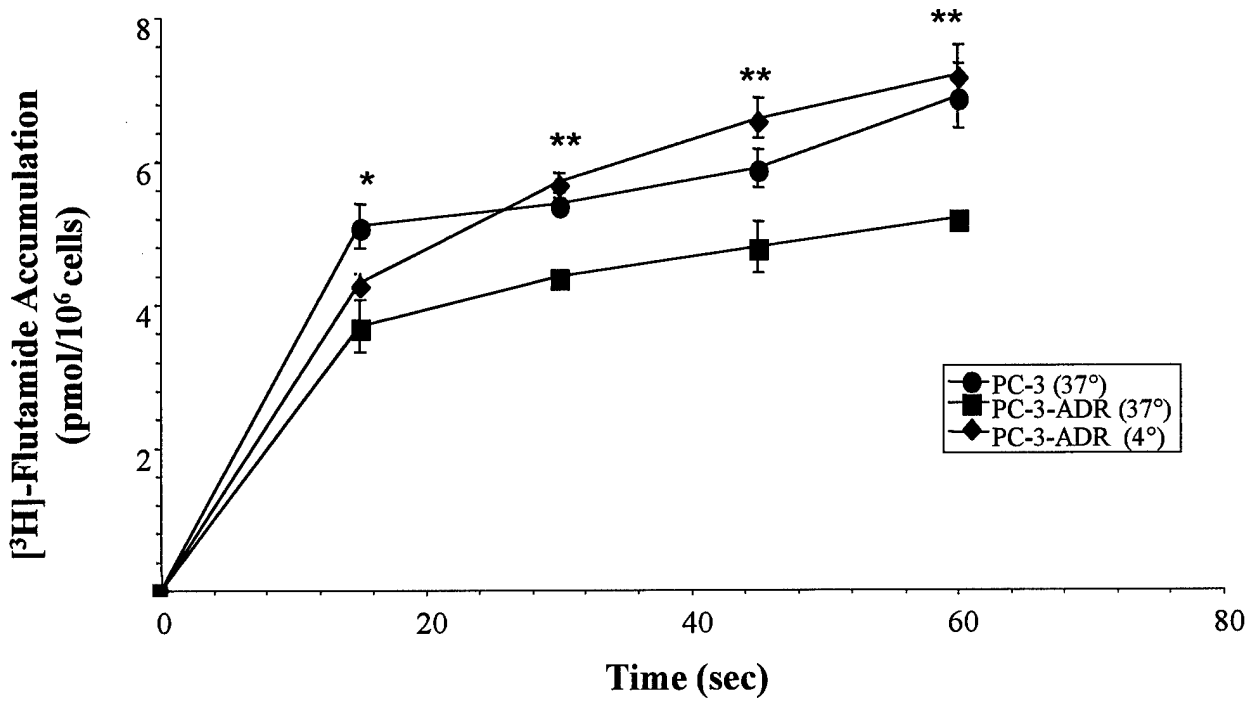
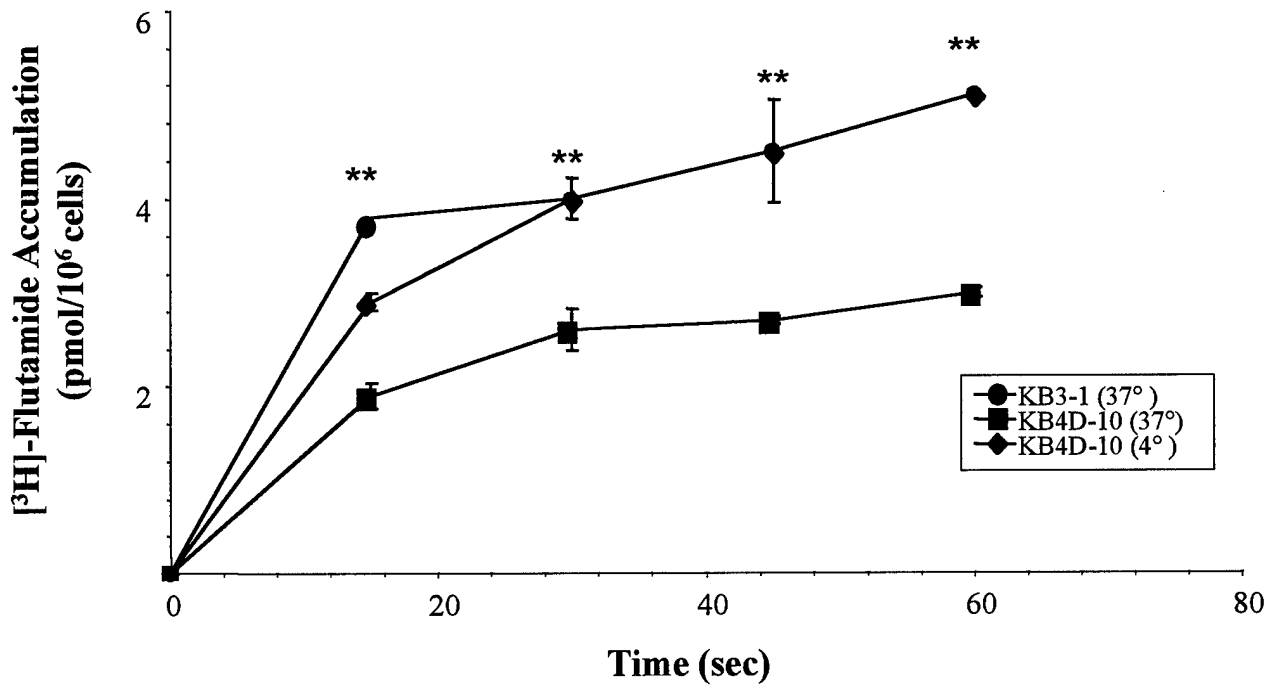


Fig. 2A

B.

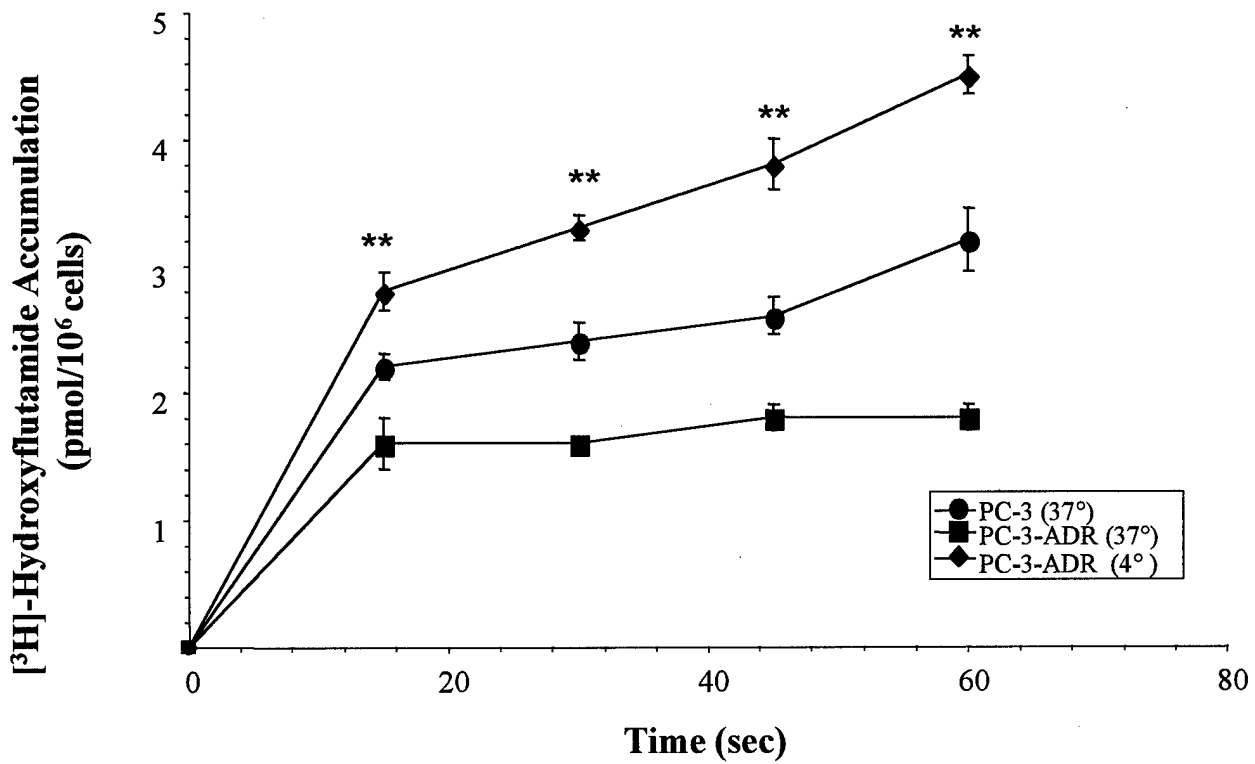
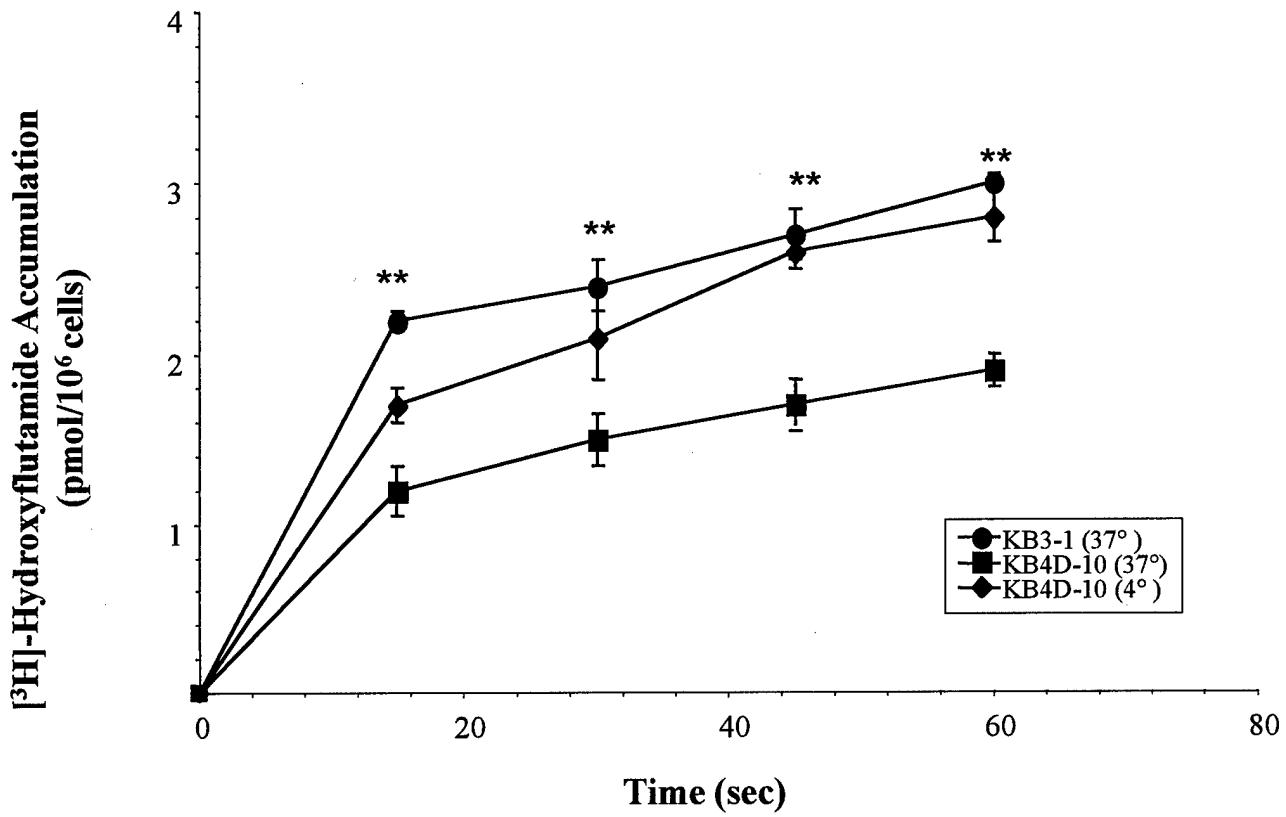


Fig. 2B

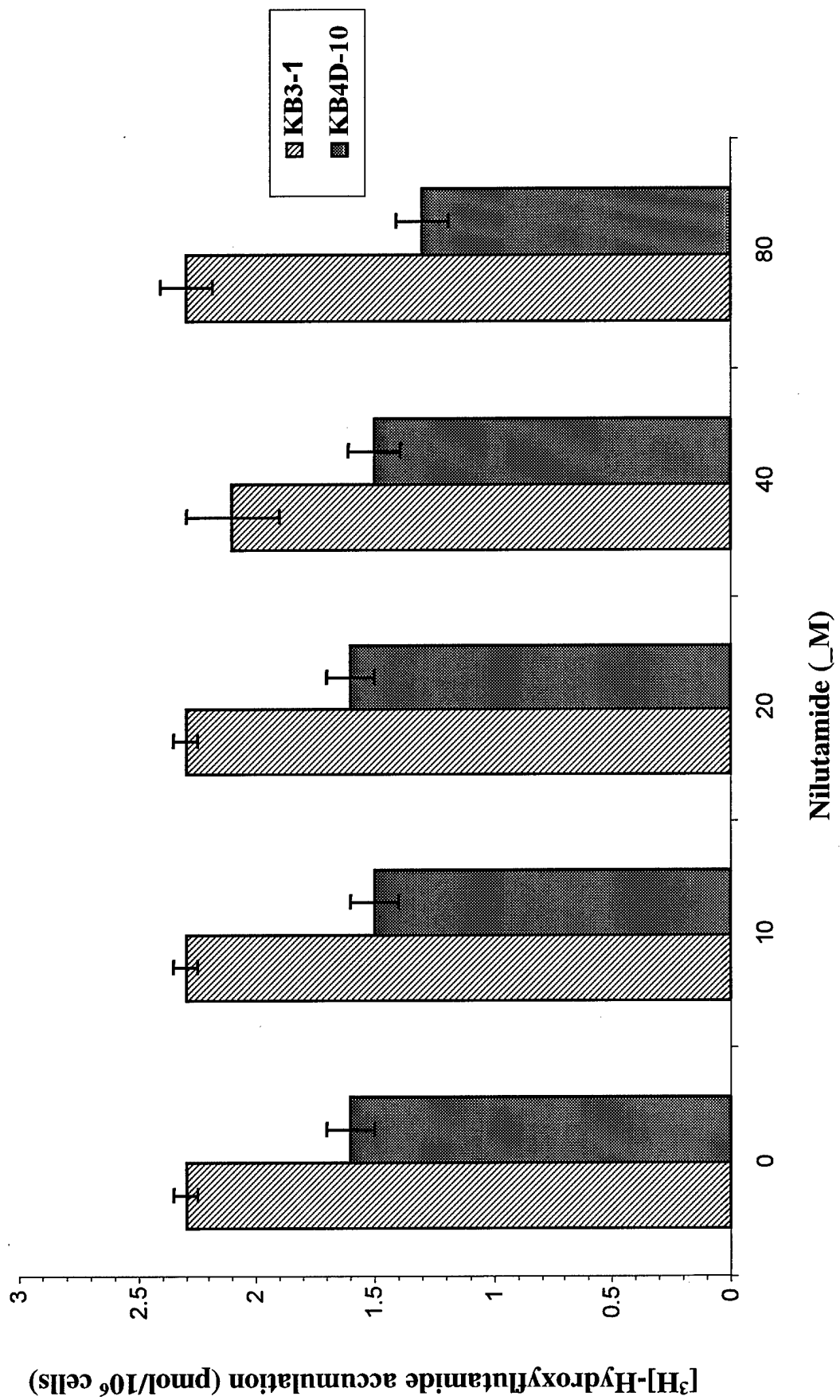


Fig. 3

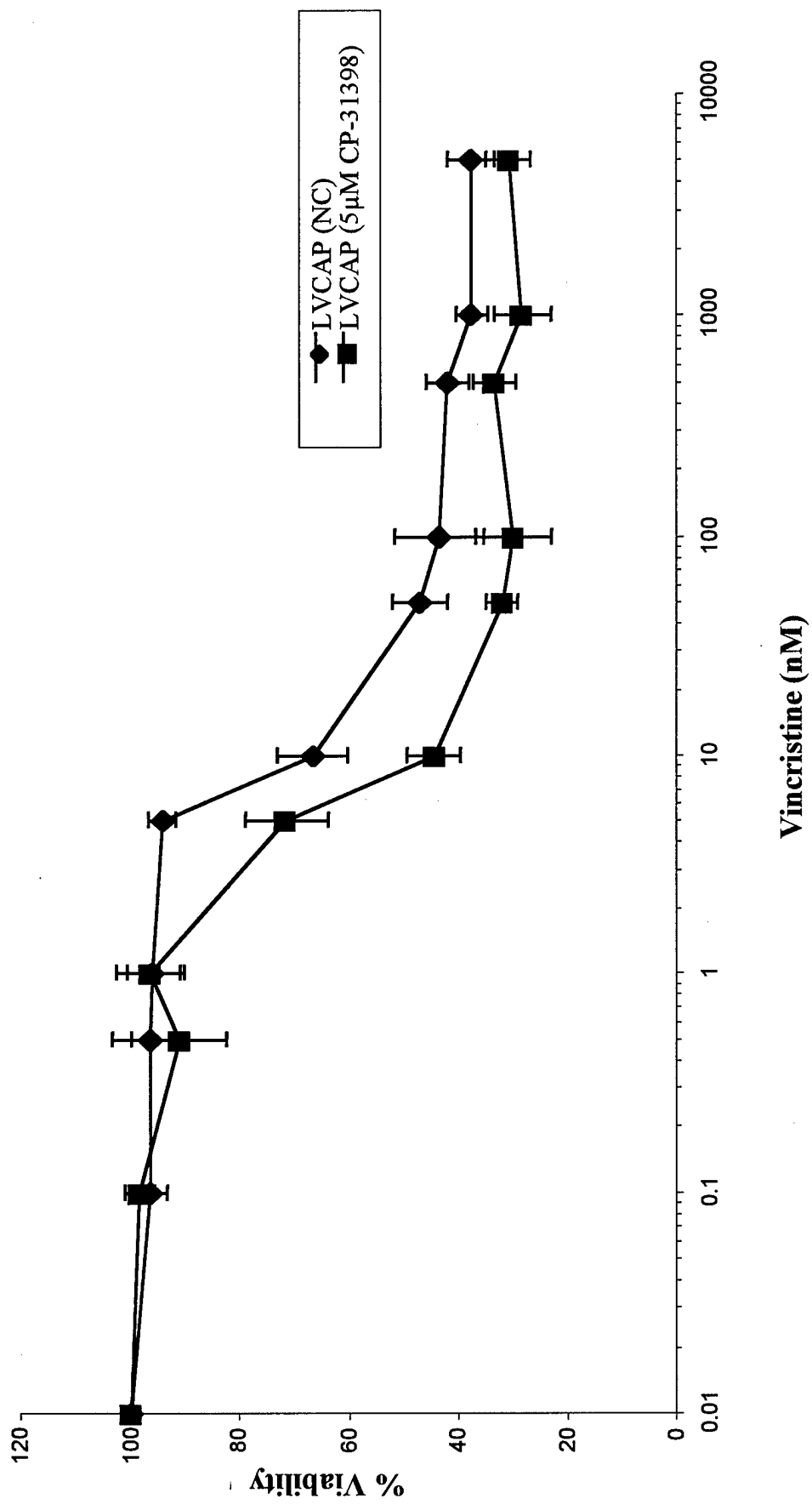
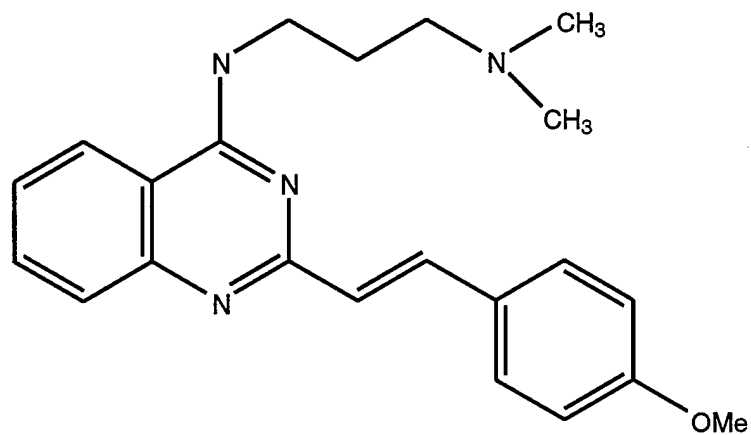
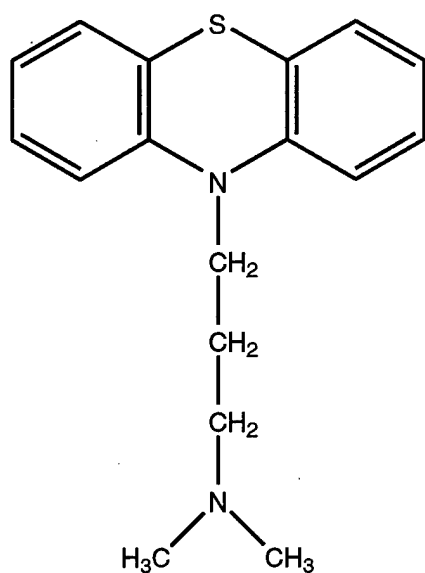


Fig. 4

CP-31398



Promazine



2-Chlorpromazine

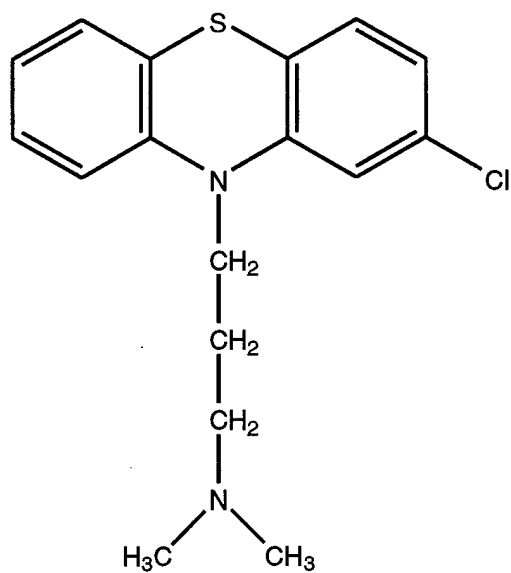
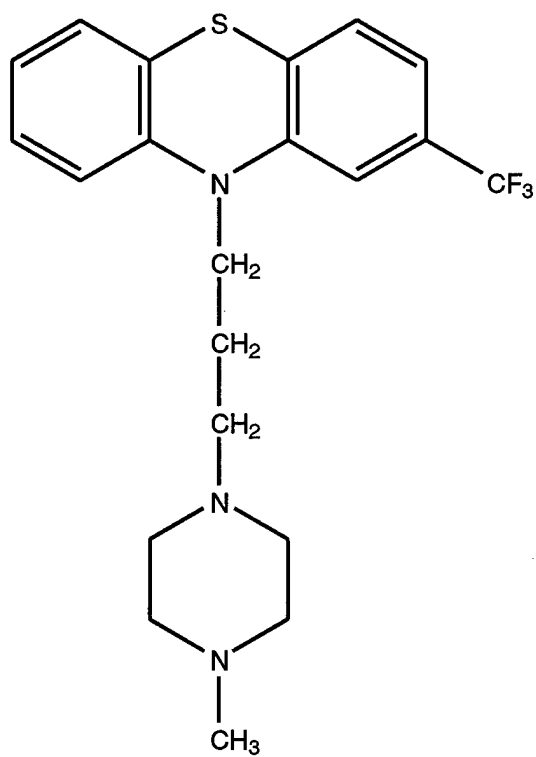


Fig. 5A

Trifluoperazine



Fluphenazine

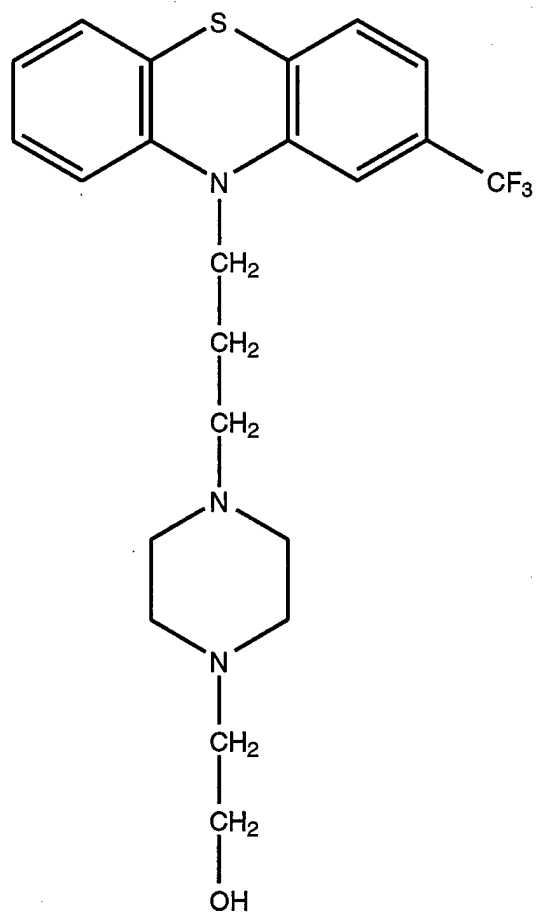
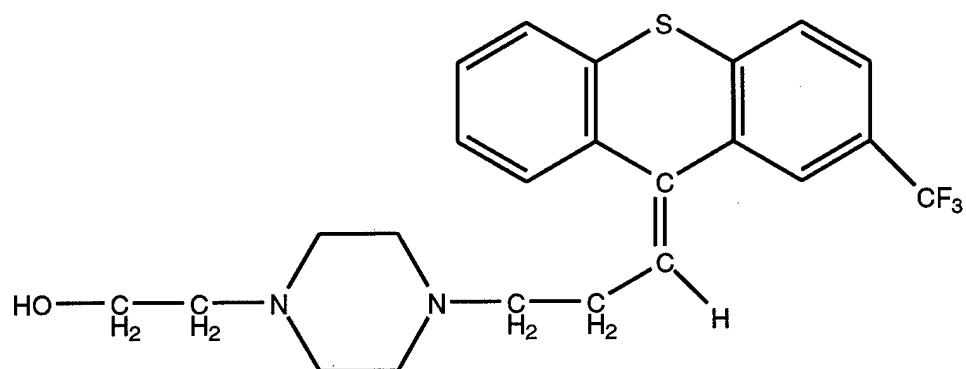


Fig. 5B

trans-Flupenthixol



Imipramine

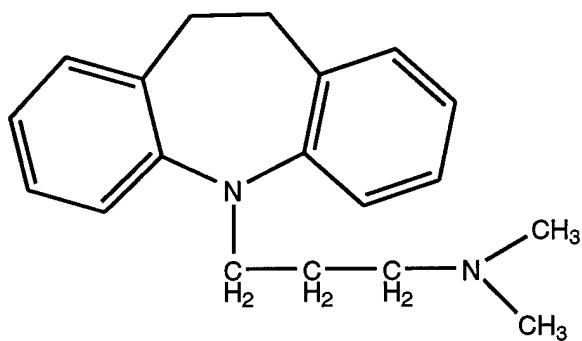


Fig. 5C

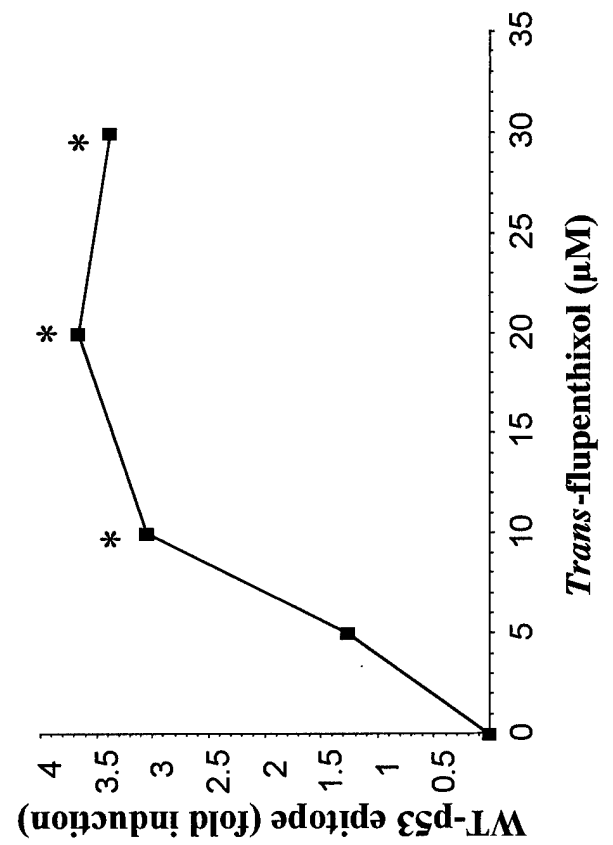
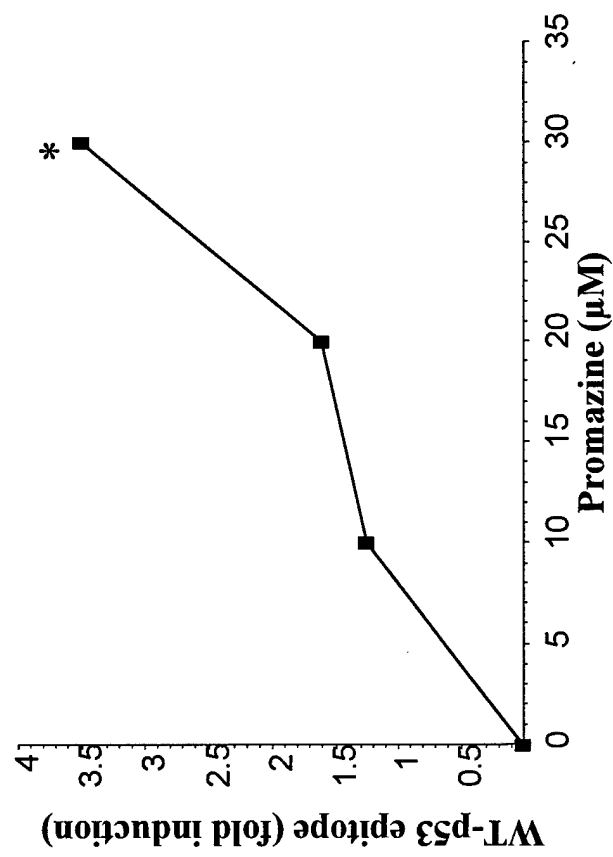
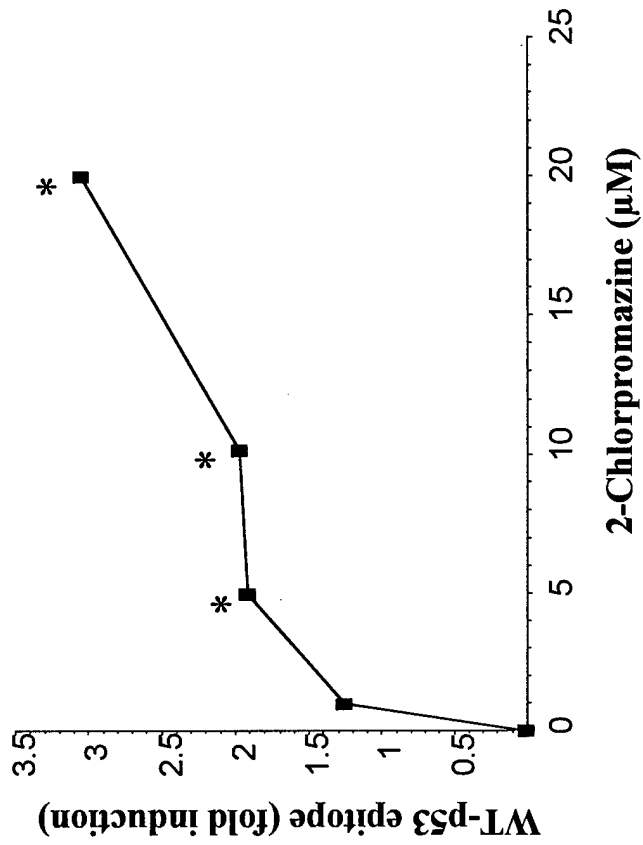


Fig. 6

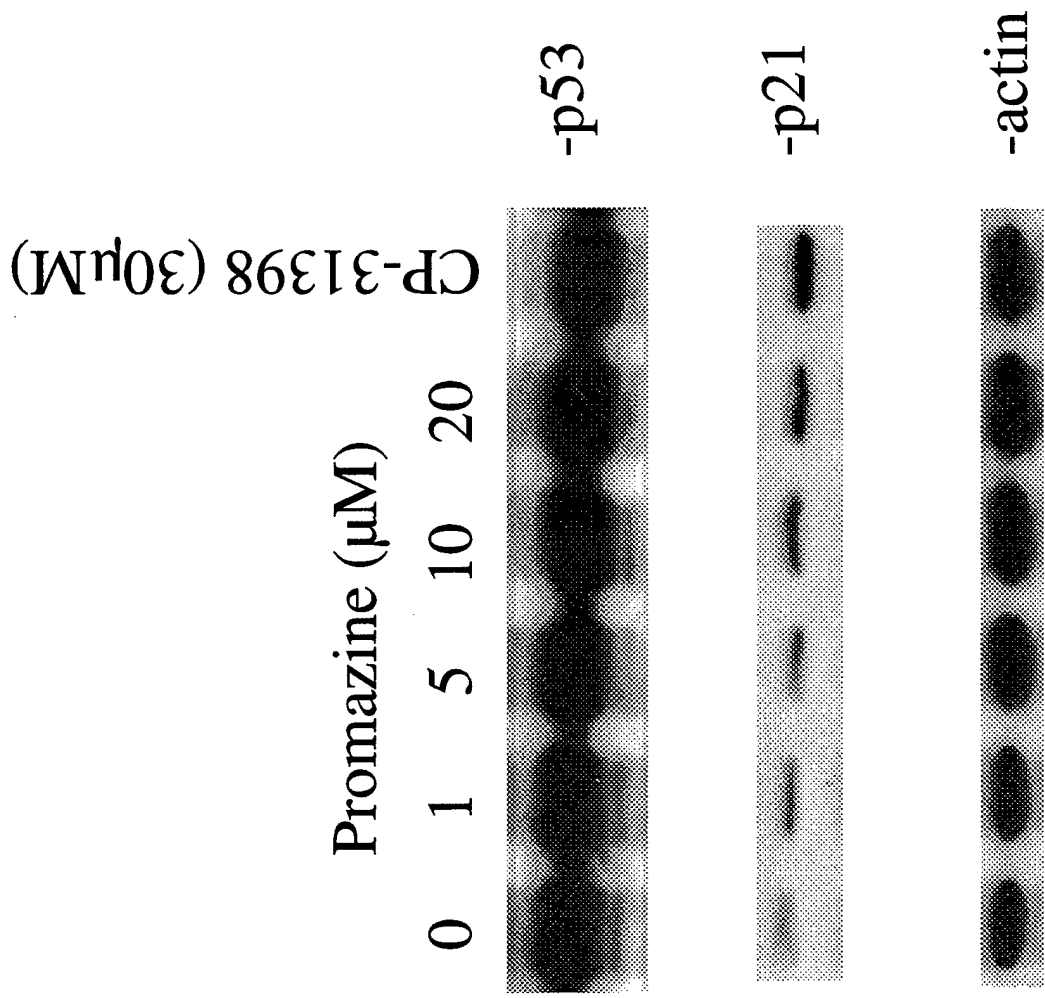


Fig. 7A

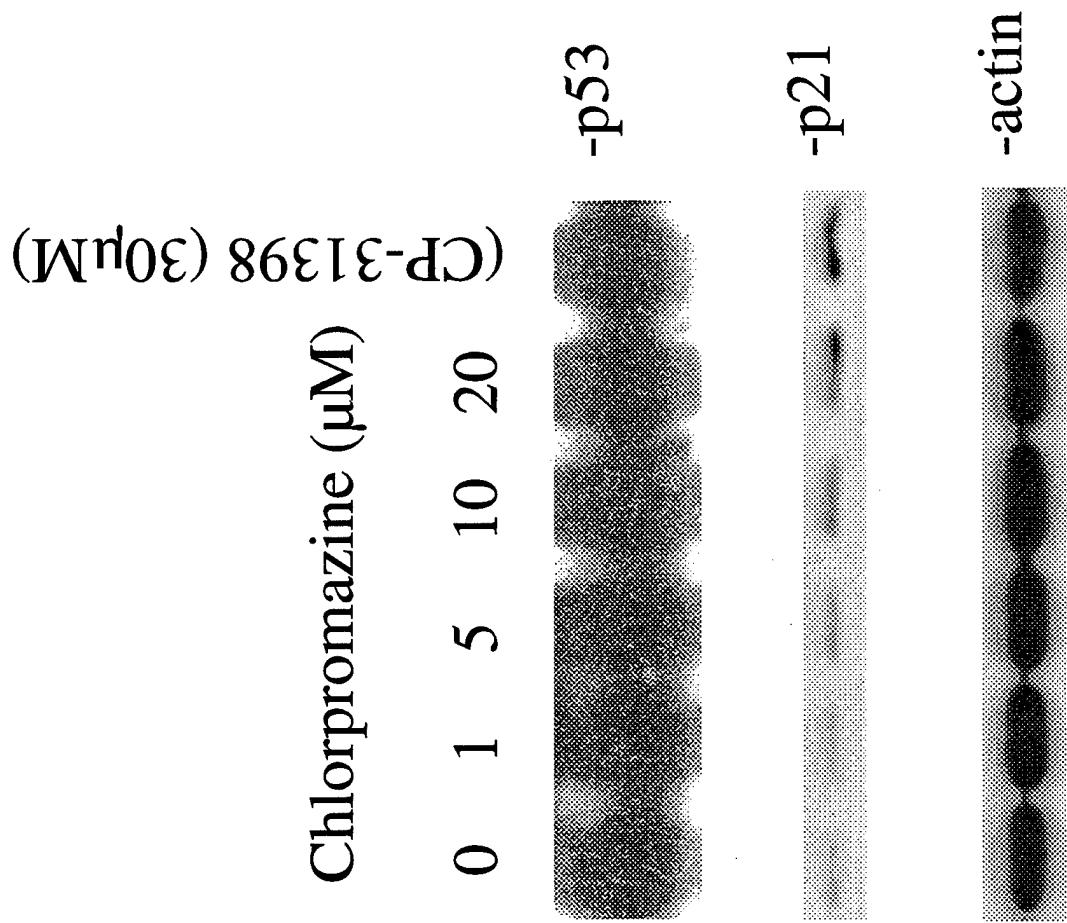


Fig. 7B

CP-31398 (30 μ M)

Trans-flupenthixol (μ M)

20 10 5 1 0

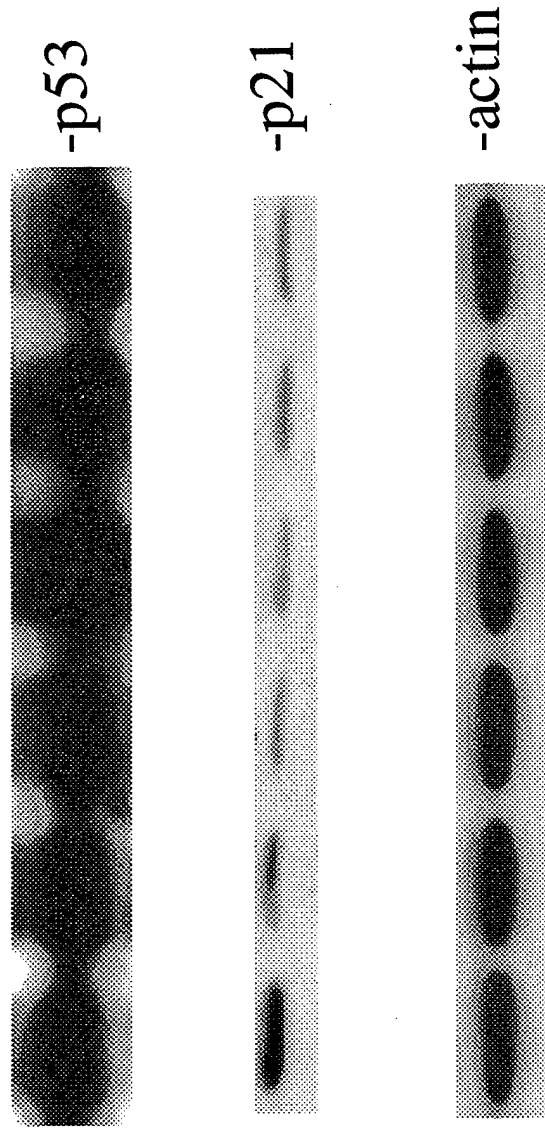


Fig. 7C